THE EFFECT OF THE ENDROT FUNGUS ON CRANBERRIES

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INTRODUCTION

The fungi which cause decay of cranberry (*Oxycoccus macrocarpus*) fruits have been the object of considerable study. Very little, however, has been written as to the chemical or morphological changes produced in the berries by any of these fungi. This is due chiefly to the practical impossibility of obtaining for inoculation cranberries which may safely be assumed to be free from fungi (5, pp. 21–23). The small size of the fruit, and the relatively slow rate at which the fungi grow render it impracticable to quarter the fruit and inoculate two portions, holding the others as controls, as was successfully done by Hawkins in peaches and potatoes (3, 4). Certain characteristics of *Fusicoccum putrefaciens* Shear are, however, so distinctive as to make it possible to secure a quantity of berries affected by this fungus and apparently free from all others.

DISTINCTIVE CHARACTERS OF GROWTH OF FUSICOCCUM PUTREFACIENS

According to Shear (6, p. 35) *F. putrefaciens* is of first importance as a cause of rot of cranberries and has been found on different varieties in Maine, Massachusetts, New Jersey, Michigan, Wisconsin, Oregon, and Washington. A very striking characteristic of this fungus in its attack on the cranberry is the fact that, so far as has been observed, decay always begins at the end of the berry. This has given rise to the name "endrot" for this disease.

The importance of endrot in Massachusetts has been pointed out by Franklin (1, p. 100; 2). In temperature studies of cranberry rot fungi it has been found that while the minimum temperature for growth of most of them is above 5° C., the endrot fungus will grow somewhat even at 0° C. (8). Unlike most cranberry fungi, *F. putrefaciens* when grown in pure culture has a bright-colored mycelium, and the fungus is thus readily identified even before it fruits (6, p. 39).

As described by Shear (6, p. 36), "Endrot first appears as a softening of the tissues accompanied by a slightly yellowish or brownish yellow watery discoloration of the skin. The diseased part is lighter-colored than the sound portion of the berry." The rot begins usually (at least in Massachusetts) at the side of the calyx and spreads until the entire inner tissue of the berry is reduced to a pulp, though the epidermis is rarely broken. The fruit thus "becomes soft and elastic to the touch, but remains turgid."

[1] The chemical work described in this paper was done by Morse, the histological work by Stevens.

Method of Selecting Material for Study

The prevalence of the endrot fungus on the Howes variety in Massachusetts, its relatively late development in the fruit, its characteristic place of attack, and its ability to grow at low temperatures, were utilized in selecting berries which were infected only by this fungus. About half a bushel of Howes, picked during September, 1917, from the state experiment bog at East Wareham, Massachusetts, were placed in storage in a crate with slatted sides and bottom so that abundant aeration was obtained. These berries were allowed to remain in storage about one month at temperatures which ranged from 15° C. to 20° C. Late in October, 1917, these berries were sorted into three lots: (1) those showing a small decayed area close to the calyx which appeared to be typical endrot, (2) perfect berries with no indication of decay, and (3) injured berries or decayed ones which did not show endrot in early stages. Lot 3 was immediately discarded, and lots 1 and 2 returned to storage, the temperature of the storeroom at this time being from 10° C. to 5° C.

On December 19, 1917, the storage lots were re-examined and material was selected for comparative study. This consisted of partly rotten berries from lot 1 (those which in October had shown incipient endrot) in which the rot had developed typically, and of sound normal berries from lot 2. Some of the berries were fixed immediately for microscopic study. Both lots were then stored at a temperature somewhat below 5° C., and portions were removed during January, February, and March. A few berries from each portion were fixed for microscopic study, and the remainder were used for chemical analysis.

Histological Observations[2]

Fusicoccum putrefaciens is evidently (Fig. 1, *A*) one of the fungi in which the hyphae often grow directly into the host cells, as distinguished from those fungi in which the hyphae grow in the intercellular spaces and either do not enter the host cells as does *Rhizopus nigricans* (7), or develop specialized haustoria which penetrate the cell walls.

While microscopic study furnished no evidence as to the way in which the fungus breaks through the cell walls of its host, there is some indication that the hyphae grow rather more readily between or within the cells than through the walls, since the hyphae frequently grow for considerable distances between the cells without breaking through and often branch at cell intersections (Fig. 1, *B* and *C*).

Moreover, the hyphae are often constricted where they pass through the cell wall (Fig. 2, *A* and *B*), and a few cases were found in which several

[2] The material for microscopic study was fixed in a solution consisting of equal parts of glacial acetic acid and absolute alcohol, imbedded in paraffin, and the sections cut from 7 μ to 10 μ thick. Several stains were used, safranin with Delafield's haematoxylin, and safranin with "light green" in clove oil proving especially useful.

hyphae entered a cell through a single opening (Fig. 2, *C*). That the hyphae readily grow through the protoplasm and into the vacuole is evident (Fig. 3, *A*), and in this case as in some others the nucleus persists and is readily distinguished after the cytoplasm is largely disorganized.

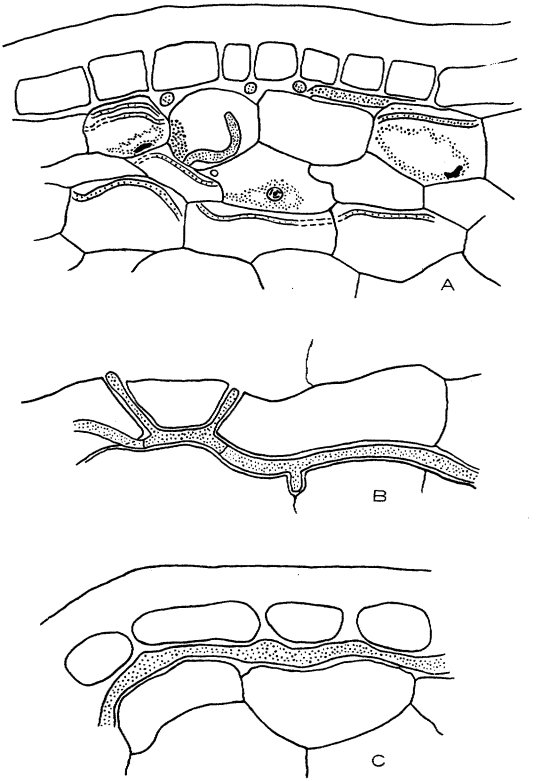

FIG. 1, *A*. Section of cranberry tissue containing hyphae of endrot fungus. Note thick cuticle, small, nearly rectangular epidermal cells, and below these the storage cells of the cranberry. Between and within the storage cells are hyphae of *Fusicoccum putrefaciens*. × 450. *B*. Section showing hyphae of *F. putrefaciens* between cells of cranberry and in three cases branching at cell intersections. × 450. *C*. Section of cranberry tissue showing single hypha between epidermal cells and subjacent storage cells. × 450.

With the apparent exception of the seeds, the fungus grows readily in all parts of the berry. Hyphae are found in the vascular bundles, as well as in the cells lining the seed cavity (Fig. 3, *A*) and in those immediately below the epidermis (Fig. 1, *A* and *C*).

In the slides examined, no case was found in which the mycelium had penetrated the cuticle, although in numerous instances the hyphae grew close to it and even under it for considerable distances. The fruiting bodies (Fig. 3 *B*) apparently develop outside the epidermal cells (the color-bearing cells) under the cuticle, which latter they finally rupture mechanically.

Histological study yielded no direct evidence as to the manner of entrance of the fungus into the berry. In the earliest stages of decay examined, hyphae were abundant in the region close to and beside the calyx. While this may be the region in which the fungus often gains entrance, it is im-

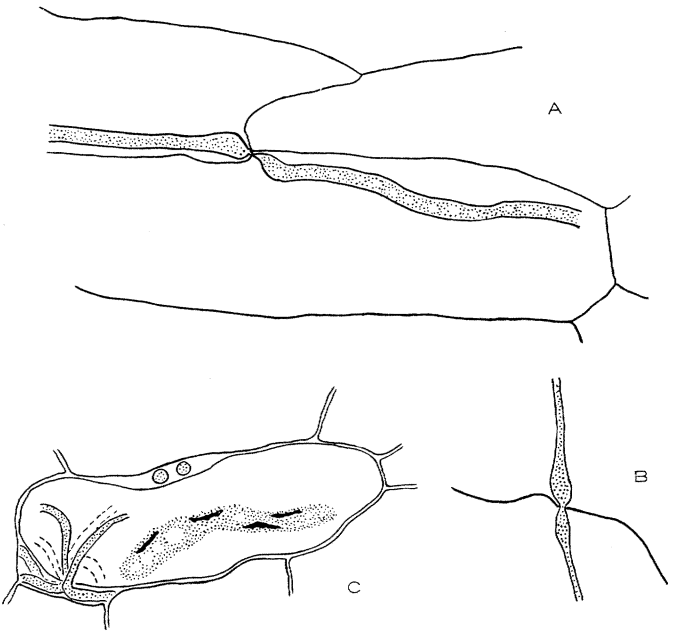

FIG. 2, *A* and *B*. Sections showing constrictions in hyphae of endrot fungus where they pass through walls of cranberry cells. × 700. *C*. Storage cell of cranberry showing several hyphae of endrot fungus entering through a single opening. × 450.

probable that this is the only place, since in Wisconsin endrot commonly is first apparent at the stem end.

CHEMICAL OBSERVATIONS

During the winter of 1918, on the dates indicated in Table 1, three samples of cranberries of the Howes variety, selected according to the method outlined above because they were infected by the endrot fungus, were received at the Massachusetts Experiment Station, Amherst, Mass. These samples had been held in storage under the conditions described, first at East Wareham, Mass., and later at Washington, D. C. In most of the berries the decay had progressed so far that the berries were very soft. One of the sound samples cited in Table 1 had been stored under the same conditions as the decayed samples.

Soon after a sample was received, duplicate charges of 50 grams each were prepared for the determination of total sugars and total acids. The remainder of the fruit was weighed and dried at a temperature of about

55° C. To promote rapid drying, it was necessary to puncture each berry in several places with a large pin, as the sound epidermis of the cranberry is nearly water-proof. The dried berries were weighed as air-dry, pulverized in a porcelain mortar, and preserved in tight bottles for subsequent analysis.

The charge for sugar and acid was mashed in a porcelain mortar, and the mass was then washed into a 500 cc. volumetric flask with about 300 cc. of water by the aid of a wash bottle and a short-stemmed funnel. The

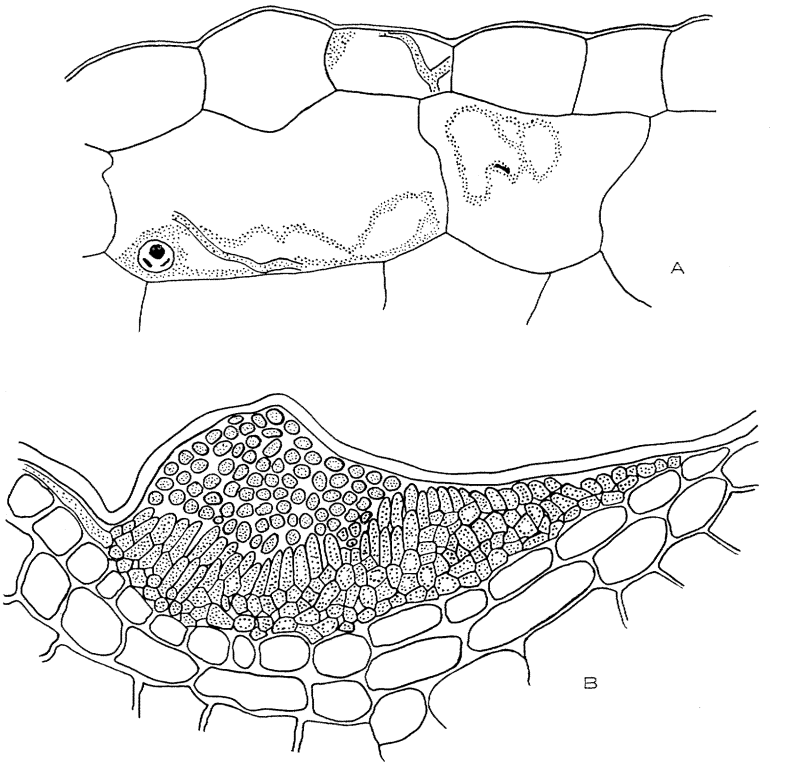

FIG. 3, *A*. Section through portion of storage tissue of cranberry adjoining seed cavity. Note slight thickening of cell walls lining the seed cavity, hyphae of *F. putrefaciens* in one of these cells lining seed cavity, and inside the protoplasm of an adjacent cell. The nucleus in this cell is still readily distinguishable. × 450. *B*. Young pycnidium of *F. putrefaciens* developing just under the cuticle of calyx. × 450.

flask was then set on the steam bath for about one hour, and the contents was repeatedly shaken so that the whole mass formed a thick liquid. After cooling the flask and contents to room temperature, the flask was filled to the 500 cc. mark and thoroughly shaken. The whole mass was thrown on a fluted filter large enough to contain it, and the funnel was covered with a watch glass while the filtrate was collected in a flask. The first 20 to 30 cc. of filtrate was thrown away.

Aliquots of the filtrate were clarified with dry lead subacetate, the excess of lead was removed by dry sodium carbonate, and the inversion and determination of the total sugars were accomplished in the usual manner.

Aliquots of the filtrate were diluted with water and titrated with standard sodium hydroxide for the total acids. The color of cranberry-juice would be expected to hide the end-point of any indicator; but as the alkali is added to the solution the color gradually changes and fades until it is a pale gray tint. A few more drops of the alkali will then produce a reasonably sharp end with phenolphthalein, which was used in all our work.

The pulverized, air-dry material was used for determinations of dry matter, ash, protein, fiber, and ether extract, which were made by the conventional methods (9).

The analytical results together with results obtained on sound berries are given in table 1.

TABLE 1. *Chemical composition of cranberries (Howes variety)*

Berries affected with endrot compared with sound fruit

Fresh Fruit

	Total Sugars Percent	Total Acid Percent	Dry Matter Percent
Rotten, January 3, 1918	2.91	2.73	11.03
Rotten, February 19, 1918	2.41	2.32	11.82
Rotten, March 5, 1918	2.83	2.25	11.64
Sound, March 5, 1918	3.40	2.21	11.82
Sound, October, 1917	3.97	2.28	12.90

Dry Matter

	Ash Percent	Protein Percent	Fiber Percent	Ether Extract Percent
Rotten, January 3, 1918	1.48	3.45	12.76	6.05
Rotten, February 19, 1918	1.47	3.54	13.90	6.13
Rotten, March 5, 1918	1.40	3.43	11.74	5.44
Sound, March 5, 1918	1.28	3.10	12.21	5.17
Sound, October, 1917	1.28	3.22	12.00	7.62

The total acid was calculated as citric acid, although the cranberry is known to contain benzoic acid, while quantitative tests showed the presence of either tartaric or malic acid or both in addition to citric. The ether extract is not true fat, but contains what is probably a wax or resin from the skin, as well as much of the acids, which are somewhat soluble in ether. The chemical determination of cellulose has not yet reached the precision required for a study like this and methods for the cellulose derivatives are still less suitable, so that only the general determination of crude fiber was attempted.

The only constituent of the fruit sufficiently affected by the rot to be manifested in the chemical analysis, is the total sugar. All the other changes in comparison with the sound fruit are apparently due to concentration as a corollary to the sugar consumption by the fungus.

Comparison of Chemical and Histological Observations

The chemical analysis of sound cranberries and of those affected by the endrot fungus shows that the only marked difference is that rotten berries contain considerably less sugar. It is apparent that the endrot fungus uses the sugar contained in the cells of the cranberry; this was to be expected from the fact that the hyphae frequently penetrate the cells and enter the vacuoles. In view of the high acid content of the cranberry and of the fact that *F. putrefaciens* is known only on that fruit, it is interesting to note that there is no considerable change in the total acid content. As the various acids known to occur were not separately determined, the possibility that the fungus uses one or more of the acids and produces some other acid in approximately equal amounts, has not been eliminated. At least one fungus producing decay of fruits has been reported to produce acid, and others have been reported as using acids (see summary in 3, p. 80). Chemical and histological observations agree in indicating that the fungus has little, if any, effect on the cuticle of the berry.

Summary

The endrot fungus has such distinctive characters that it was possible to select cranberries rotted by this fungus alone. A histological study of those selected berries showed that the endrot fungus grows in all parts of the berry except the seeds and the cuticle and is able to penetrate cell walls and protoplasm. A chemical study showed that the sugar content of berries rotted by the endrot fungus is much lower than that of sound fruit. The fungus thus apparently utilizes these sugars.

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